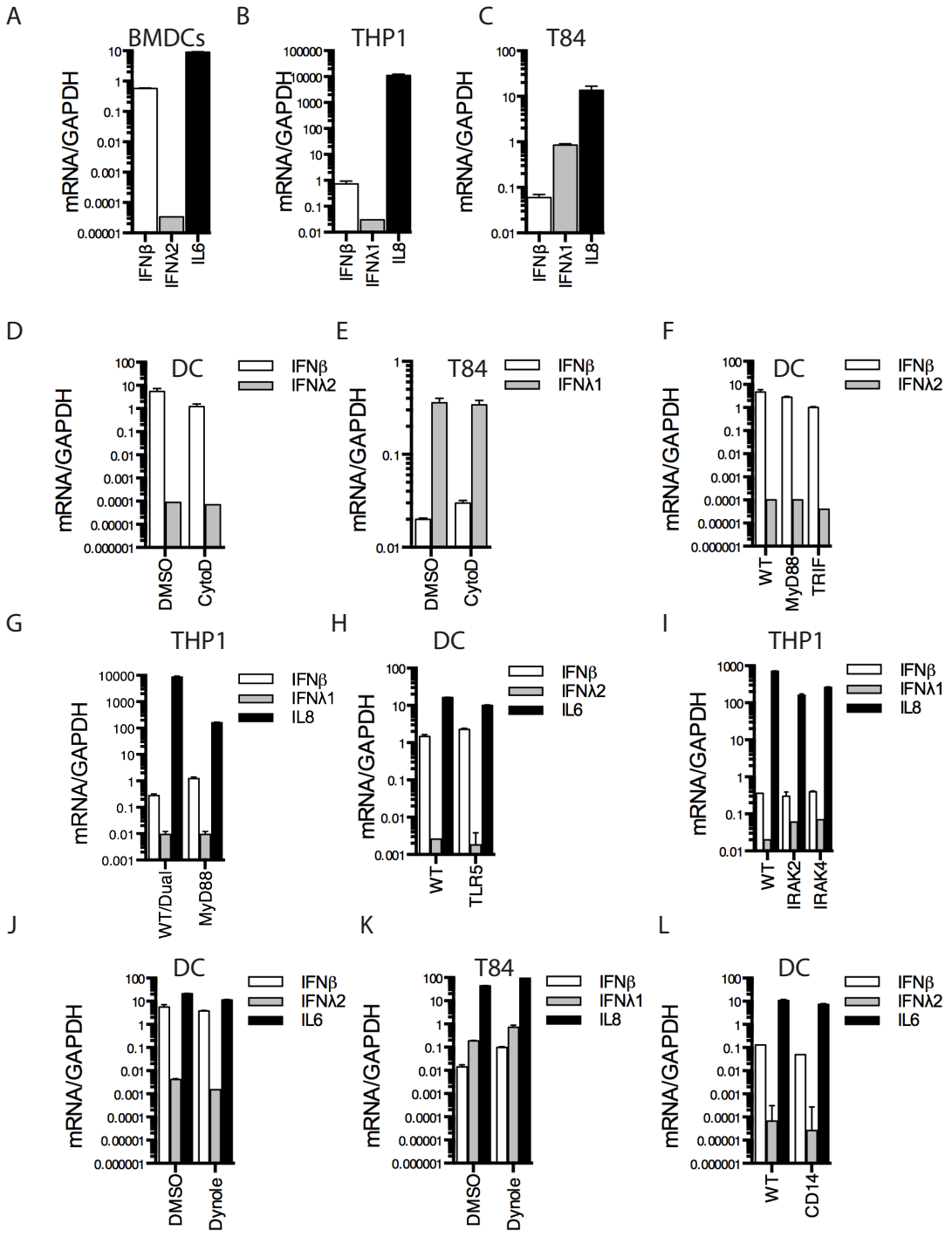
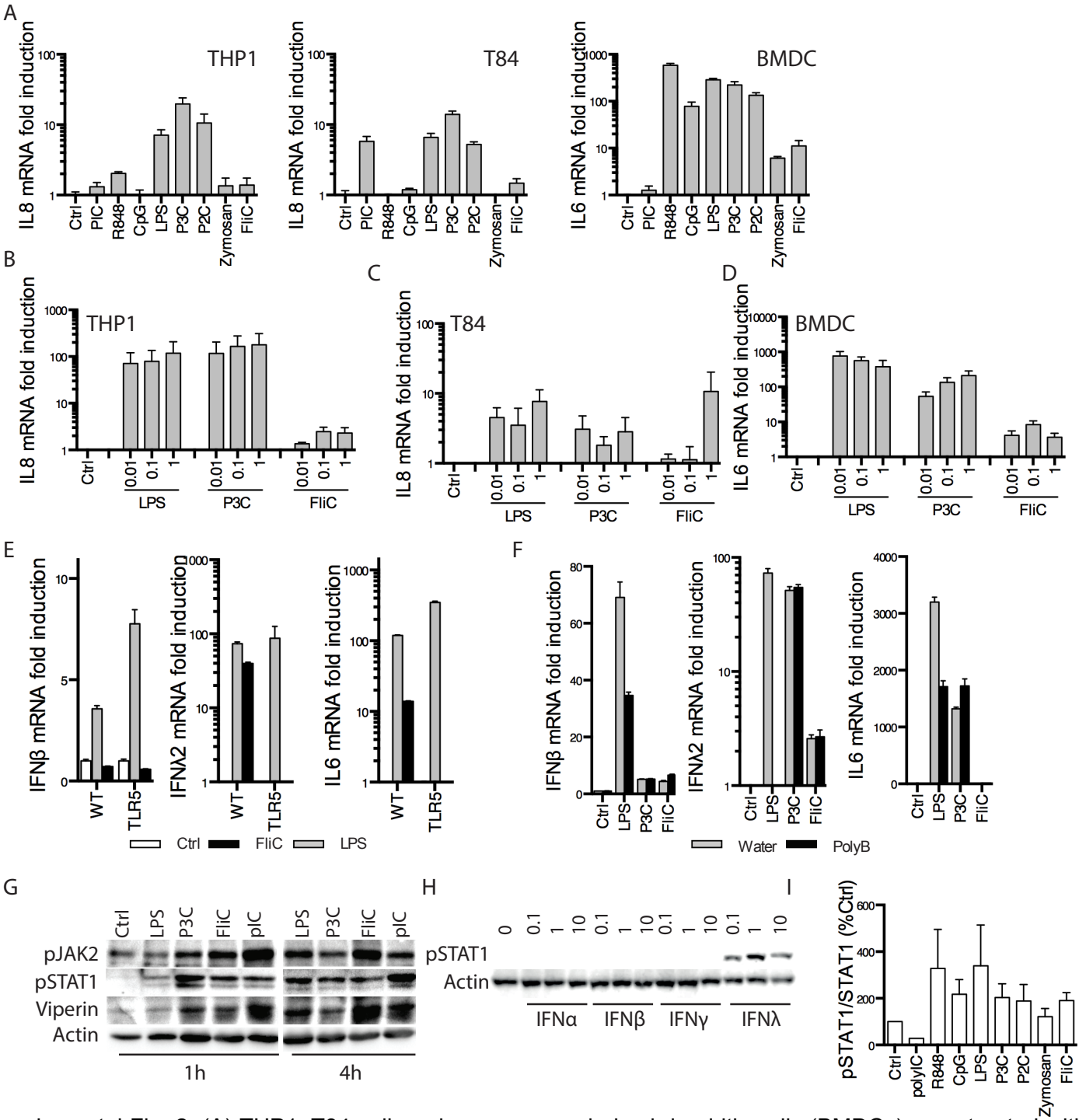


Supplemental Fig. 1: As indicated IFNβ, IFNλ1, IFNλ2, IL6 or IL8 mRNA levels were quantified in untreated cells and normalized to GAPDH levels: (A) WT Bone Marrow derived Dendritic cells (BMDCs), (B) PMA-activated human THP1 monocytes, (C) polarized T84 cells, (D, E) DMSO or Cytochalasin D (CytoD) treated BMDCs or polarized T84s, (F), WT, MyD88 or TRIF KO BMDCs, (G), WT/Dual control or MyD88 KO THP1 cells, (H) WT or TLR5 KO BMDCs, (I) WT, IRAK2 or IRAK4 deficient THP1 cells, (J, K) DMSO or Dynole-treated BMDCs or T84 cells, (L) WT or CD14 KO BMDCs. Data shown are one experiment representative of 2-10.



Supplemental Fig. 2: (A) THP1, T84 cells or bone-marrow derived dendritic cells (BMDCs) were treated with 10 µg/ml polyIC (pIC), 0.1 µg/ml R848, 0.1 µg/m CpG DNA, 0.1 µg/m LPS, 0.01 µg/ml Pam3Cys (P3C), 0.01 µg/ml Pam2Cys (P2C), 1 µg/ml Zymosan or 0.1 µg/ml flagellin (FliC) for 5 h. (B) THP1 cells, (C) T84 cells or (D) BMDCs were challenged with the indicated concentrations of LPS, P3C or FliC for 5 h. IL8 or IL6 mRNA expression was measured by RT-qPCR. (E) WT or TLR5 KO BMDCs were challenged with LPS or FliC for 5h. (F) LPS, P3C or FliC were treated with water or the antibiotic PolymyxinB (polyB) for 30 min. WT BMDCs were challenged with the ligands, as indicated. IFNβ, IFNλ2 or IL6 mRNA expression was measured by RT-qPCR. (G) T84 cells were challenged with 0.1 µg/ml LPS, Pam3Cys or FliC or 10 µg/ml polyIC (pIC) for the indicated time points. STAT1 and JAK2 phosphorylation (pSTAT1, pJAK2), as well as viperin expression were analyzed by western immunoblotting, using actin as a loading control. (H) 0.1, 1, 10 ng/ml mouse IFNα, IFNγ, IFNβ and IFNλ2 was incubated onto human 293T cells for 1h, STAT1 phosphorylation was monitored by western immunoblotting using actin as a loading control. (I) Mouse BMDCs were challenged with 10 µg/ml PolyIC (pIC), 0.1 µg/ml R848, 0.1 µg/ml CpG DNA, 0.1 µg/ml LPS, 0.01 µg/ml P3C, 0.01 µg/ml P2C, 1 µg/ml Zymosan or 0.1 µg/ml FliC for 5 h. Cell culture supernatants were collected and applied onto 293T cells for 1h. As a marker of type III IFN activity, STAT1 phosphorylation was analyzed by western immunoblotting, with total STAT1 and actin used as loading controls (see Fig. 3J). Densitometric analyses were carried out using ImageJ. pSTAT1 band density over STAT1 loading control was quantified and normalized to untreated control (Ctrl).

Each panel represents one experiment representative of at least 2 (A, E-H) or averages ± SEM of 3-6.